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Note

Analysis of pentachlorophenol in waste water using high-performance liquid chromatography

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For the past thirty years, pentachlorophenol (PCP) has been used extensively as a herbicide, fungicide and insecticide, mainly for the preservation of wood and wood products. According to Erstand[1] over 40,000 tons of PCP are being used annually in the U.S.A. This extensive use has caused concern among many in our pollution-conscious society mainly because of the toxic properties of this chemical and because of the environmental problems with other chlorinated and brominated compounds.

It has long been known that PCP is a toxic agent not only to the wood destroying organisms, but also to human beings and other animals[2,3]. Unfortunately there is still a considerable amount of controversy over the amount of residual PCP in the environment, the rate of biodegradation and the long terme effects of this material on animals.

Unligil[4], Cserjesi[5–7] and others[8,9] have identified naturally occurring rot fungi which are capable of detoxifying PCP. Other studies have shown that sunlight can degrade PCP[10,11]. On this basis it has been assumed that PCP is not persistent in the environment but is readily degradable in both water and soil.

Whether or not this is the case has yet to be decided. Other researchers have found substantial amounts of PCP in the environment. Crammer and Freal[12] found PCP in the range of 2.2 to 10.8 ppb (10^9) in the urine of humans whom they considered to be representative of the general population. According to Bevenue *et al.*[13] PCP is also present in municipal water supplies, wells and paints. Recent studies by Buhler *et al.*[14] also confirmed the presence of PCP in municipal sewage, river water and treated river water.

Efforts are being made by the Environmental Protection Agency to control the amount of PCP being expelled into the environment by wood-treating industries. In order to do this, reliable and relatively simple methods for analysis are needed. The colorimetric procedures, which are used for PCP analysis[15], will also give similar color reactions with many other phenolic compounds. Column and thin-layer techniques have also been developed for separating PCP[16–18]. However, these methods are not suitable for low-level analysis of PCP. The best method for determining PCP is conversion into the methyl ether followed by analysis using gas chromatography

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(GC) with an electron-capture detector, or GC coupled with mass spectrometry (MS)[19]. Both of these methods require an extensive amount of pre-treatment and highly trained personnel for the operation of the equipment[14,20,21].

The objective of this study was to develop a high-performance liquid chromatographic (HPLC) method for determining low concentrations of pentachlorophenol. Technical-grade PCP, which is the grade most commonly used in the wood treating industry, is only about 85–90% PCP. The other major component is 4–8% 2,3,4,6-tetrachlorophenol. Besides the major components, there are also traces of mono-, di- and trichlorophenols, octa-, hepta- and hexachlorodibenzo-*p*-dioxins, and a variety of other polychlorinated aromatic compounds[11]. Consequently any HPLC method which is developed must be capable of separating not only the products which come from the wood treating process (*e.g.* hydrocarbons and wood extractives), but also the components found in technical-grade PCP.

EXPERIMENTAL

A Waters Assoc. (Milford, Mass., U.S.A.) Model 202/401 liquid chromatograph equipped with a 5000-p.s.i. pumping system with an ultraviolet detector (Waters Assoc., Model 202 or 440) and differential refractometer detector (Waters Assoc., Model R-400) was employed. All chromatograms were made at room temperature and at a constant flow-rate. The pre-packed microparticulate silica gel column (No. 6504-044) was obtained from Whatman (Clifton, N.J., U.S.A.). All the solvents used in this study were reagent-grade solvents. Immediately before use, the solvent was dried by passing through a column of dry, porous activated silica (Davison grade 35 silica, 12–42 mesh) followed by filtration through a 0.5-μm filter. The operating parameters used in this separation were column: 25 cm $\times$ 4.6 mm I.D.; flow-rate: 0.54 ml/min; particle size: 10 μm (irregular shape).

A 50-ml volume of each of the waste water samples was first acidified with a solution of 4 *N* sulfuric acid to a pH of 3–5. Each sample was then extracted with 20 ml of chloroform three separate times. The chloroform extract was dried over anhydrous sodium sulfate. The chloroform-soluble waste water components were concentrated to dryness by rotary evaporation without heat. Earlier studies[22] had shown that no loss of pentachlorophenol occurred at this step providing the temperature was kept below 30°. The waste water samples were dissolved in 10 ml of chloroform and analyzed directly by HPLC. The samples which were found to contain less than 1 ppm of PCP were repeated using the same procedure, except the starting volume was 100 ml and the sample used for final HPLC analysis was dissolved in 2 ml of chloroform. All samples were run in duplicate. Three types of water samples were analyzed: "incoming water" which was the water coming into the plant before it was used in the treating process, "untreated waste water" which was the water obtained directly after it had come from the treating cylinder, and "treated waste water" which had undergone some type of primary treatment. All the samples were analyzed within 24 h after collection.

RESULTS AND DISCUSSION

A variety of solvent combinations were evaluated in order to separate the

components in technical-grade PCP on a microparticulate silica gel column. The first solvent which separated PCP from the other chlorinated phenols present in technical-grade PCP was cyclohexane–acetic acid (98:2, v/v). The type of separation obtained using this combination is shown in Fig. 1. The first peak, as determined by GC–MS analysis, consisted of a complex mixture of polychlorinated compounds, including octa-, hepta- and hexachlorodibenzo-*p*-dioxins, as well as a variety of other polychlorinated ethers and furans. The second peak contained a mixture of products including 2,4,6-trichlorophenol, the third peak was mainly 2,3,4,6-tetrachlorophenol, and the fourth peak was PCP.

A linear calibration curve was obtained when peak heights were plotted *versus* concentrations of PCP using a fixed-wavelength detector (254 nm) (Fig. 2). The minimum concentration of PCP which can be detected without concentrating the sample is 1.0 ppm. For repeated injections of the same sample, the precision (coefficient of variation) was 1–2% using a standard solution of PCP.

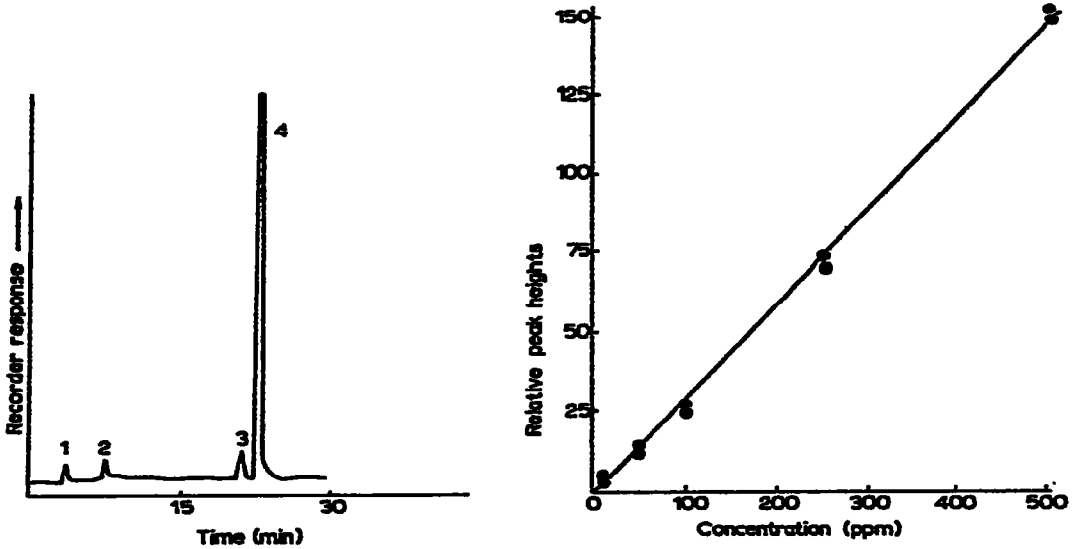

Fig. 1. Separation of technical PCP using cyclohexane-acetic acid (98:2) as the eluting solvent. Peaks: 1 = mixture of dioxins and other polychlorinated products; 2 = mixture of chlorinated phenols including trichlorophenol; 3 = tetrachlorophenol; 4 = PCP.

Fig. 2. Relationship between peak areas and concentration of PCP.

Results obtained by the HPLC method were compared to a GC–MS procedure[19]. The samples used in this study were water samples taken from wood treating plants located throughout the U.S.A. and were part of an Environmental Protection Agency study. The results of this study are shown in Table I. In general, good agreement was found between the MS and HPLC procedures, except for samples 2, 3 and 6.

A variety of other solvent combinations were found which could also be used to separate the components in technical-grade pentachlorophenol. All of these solvent systems could be used to separate pentachlorophenol, the major component of

TABLE I

TYPICAL ANALYSES OF PENTACHLOROPHENOL FROM WOOD TREATING PLANTS

No.	*Source of sample*	*Concentration (HPLC) values (ppm)*	*Concentration (MS) values (ppm)*
1	Treated waste water	72.2	75.0
2	Untreated waste water	17.5	32
3	Untreated waste water	13.5	18.0
4	Treated water	9.9	9.7
5	Treated waste water	9.1	6.4
6	Untreated waste water	8.2	17
7	Treated waste water	4.7	4.4
8	Treated waste water	4.6	4.0
9	Treated waste water	4.4	3.9
10	Treated waste water	4.3	4.1
11	Treated waste water	0.33	0.29
12	Treated waste water	0.29	0.16
13	Incoming water	0.25	0.14
14	Treated waste water	0.027	0.20
15	Incoming water	0.011	>0.010

TABLE II

PENTACHLOROPHENOL RETENTION TIMES WITH DIFFERENT ELUTING SOLVENTS

Solvents	*Proportions (%)*	*Retention time (min)*
Cyclohexane–acetic acid	98:2	24:5
Cyclohexane–methylene chloride*	57:43	9:7
Hexane–methylene chloride*	90:10	17:7
Hexane–methylene chloride*	80:20	10:0
Hexane–methylene chloride*	60:40	8:3
Hexane–acetic acid	98:2	22:7
Hexane–acetic acid	96.7:3.3	19:2
Hexane–acetic acid	95:5	12:1
Hexane–chloroform*	95:5	18:6
Cyclohexane–chloroform	80:20	15:0

* Contains 1% acetic acid.

technical PCP. The relative retention times of PCP using these solvent systems are given in Table II.

In summary, an HPLC procedure has been developed for separating the components found in technical-grade PCP. This procedure can also be used for determining the concentrations from plant effluents with a minimum of sample preparation.

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